Docket No.: 45,394

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Schlom, et al.

Serial No.: 08/500,306 Examiner: Bansal, G.

Filed: July 10, 1995 Group: 1642

For: GENERATION OF IMMUNE RESPONSES TO PROSTATE-SPECIFIC ANTIGEN (PSA)

DECLARATION

I, Jeffrey Schlom, hereby declare:

I am a co-inventor of the above-described application.

I am Chief, Laboratory of Tumor Immunology and Biology, Division of Basic Science, National Cancer Institute, National Institutes of Health, Washington, D.C. In this capacity, I coordinate a variety of clinical testing that is carried out in conjunction with my lab. One of the areas where I have coordinated and reviewed such clinical testing involves the use of recombinant pox viruses expressing PSA to induce an immune reaction in individuals suffering from prostate cancer. Clinical trials in this area have been performed at the Dana-Farber Cancer Institute, Boston, Massachusetts. The clinical investigator at Dana Farber Cancer Institute that I am working with is Dr. Donald Kufe.

A clinical protocol was performed where three different dose ranges of a recombinant pox virus expressing PSA were tried with two boosts given at monthly intervals (see Appendix "A"). A low dose (2.65×10^6pfu of recombinant vaccine expressing PSA, rV-PSA), a medium dose (2.65×10^7pfu rV-PSA), a high dose (2.65×10^8pfu rV-PSA),

and a high dose with 250 μg/m² GM-CSF. The reason for this is that as with all human testing, the first set of tests are primarily carried out to determine the maximum safe dose.

However, other data in the patients being tested is also looked at. Among such data are generation of immune response and PSA levels.

Although individuals with prostate cancer generally display levels of PSA that continue to increase over time, in the individuals tested with recombinant pox expressing PSA (rV-PSA), the PSA levels were stable in a significant number of the individuals.

Specifically, in 14 of the 33 individuals the PSA levels were stable for a period greater than six months, 9 patients remained stable for 11-25 months. In fact, in individuals receiving the highest dose, plus GM-CSF, a significant number of the patients had PSA levels that were stable for at least 6 months (see attached Figure).

The finding that administration of the recombinant pox virus-PSA construct results in stabilizing PSA levels, which otherwise ordinarily increase, despite being a self-antigen t is something that those of ordinary skill in the art would not have expected. I have encountered considerable skepticism from those of ordinary skill in the art that a self antigen such as PSA could generate a sufficient immune response to inhibit tumor growth. Consequently, it is my opinion that this finding would be unexpected to those of ordinary skill in the art.

Our data also confirms that an immune response is seen as a result of the administration and boosting of the pox virus-PSA construct.

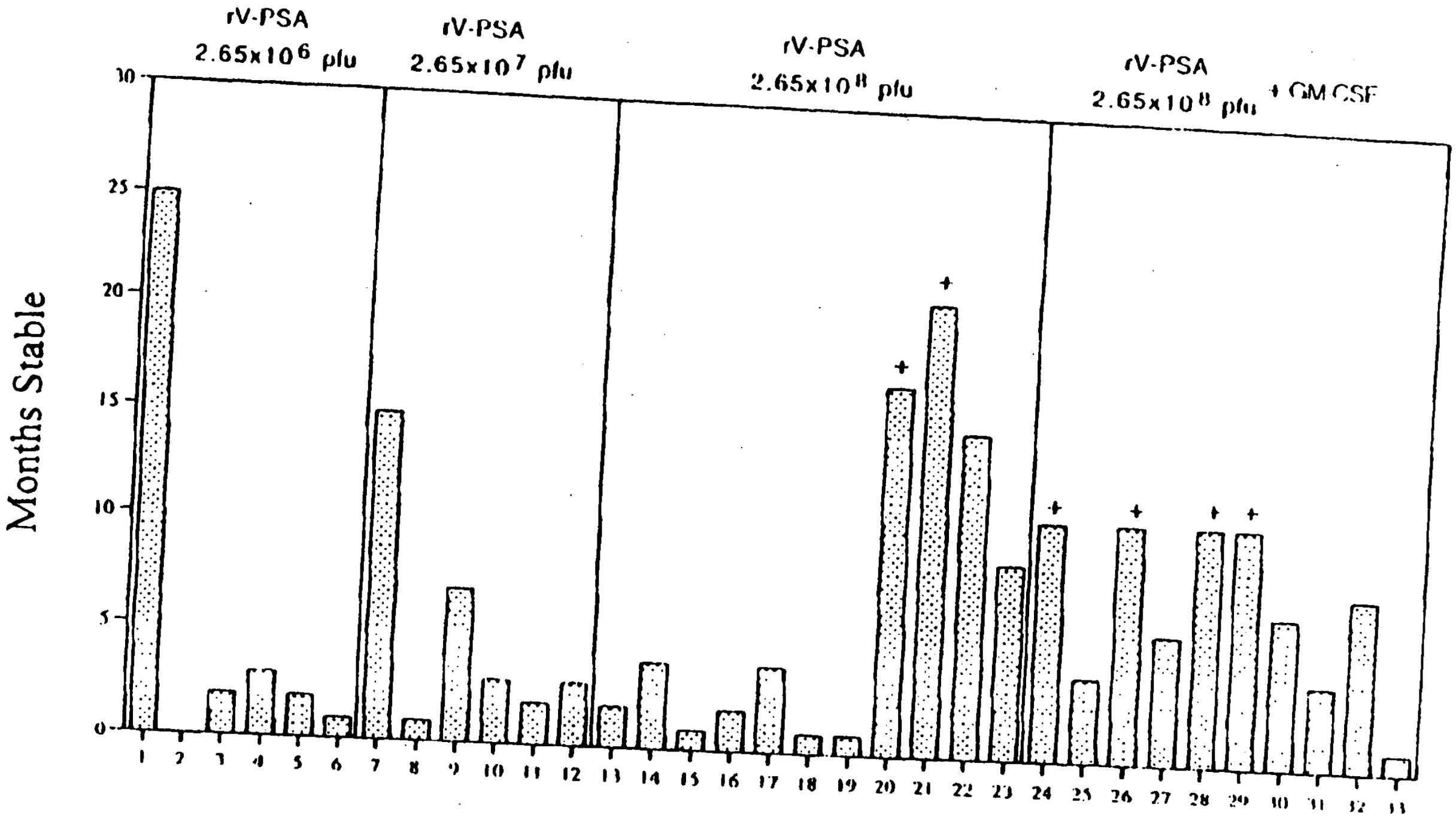
I hereby declare that all statements made herein are of my own knowledge are true and that all statements made on information are believed to be true, and further that these

statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, and that such willful false statements may jeopardize the validity of the application or any patent that issues therefrom.

Respectfully submitted,

Jeffrey Schlom

APPENDIX "A"



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DECLARATION UNDER 37 CFR 1.131

We, Jeffrey Schlom and Dennis Panicali, hereby declare as follows:

1. We are co-inventors of the above-described application.

2. We are aware that the Examiner has cited Spitler et al. (WO95/04548). This application has an international publication date of February 16, 1995 and a U. S. Priority date of August 11, 1993. A patent issued corresponding to this PCT publication issued as U.S. Patent No. 5,925,362.

3. Prior to August 11, 1993, we had completed in the United States a recombinant pox virus expressing prostate specific antigen (PSA). Two recombinant vaccinia viruses of the Wyeth strain were prepared, vT119 and vT1001. In vT119, the PSA gene is inserted at the Hind III J insertion site (i.e., the tk gene site), whereas in vT1001, the PSA gene is inserted at the Hind III M insertion site (see pages 1-2 of "Quarterly Progress Report Contract #NO1-CB-21154-02 Production of Recombinant Vaccina Virus Expressing Prostate Specific Antigen (PSA)", attached hereto as Exhibit "A". The date of this Progress Report has been redacted but was prior to August 11, 1993.

4. We made the PSA pox constructs because we had conceived in the United States prior to August 11, 1993, the idea of using a recombinant pox viral vector having an insertion site containing a DNA segment encoding PSA operably linked to a promoter and capable of expression in a host as a cancer vaccine for people suffering from prostate cancer.

5. We conceived in the United States prior to August 11, 1993, that by administration of that pox virus expressing PSA in an individual having such prostate cancer, one would stimulate the immune system, specifically both the humoral and cellular response to PSA in such individuals. The cell-mediated response would include a cytotoxic T Lymphocyte (CTL) response.

6. Consequently, we prepared the aforementioned recombinant pox vector expressing PSA to use in that method. (See Exhibit "A").

7. As taught therein, we prepared vaccinia vectors containing the PSA gene and confirmed that vT119 expressed PSA. Then we began work on preparing a master stock. (Exhibit "A" at p.2).

8. These steps were necessary to make the viral vector in sufficient quantities, to be able to perform animal testing, which had to be performed to show that such vector was safe, before we could engage in the ultimate goal, human tests.

9. A copy of an initial work plan discussing our scientific programs that listed under "The Cancer Vaccine Program" the PSA vaccinia recombinant is attached as Exhibit "B." Other cancer vaccines that we were looking at have been redacted as has the date.

The date of the work, which was performed in the United States, is prior to August 11, 1993.

10. Thus, prior to August 11, 1993, we had conceived in the United States utilizing PSA in a recombinant pox virus vector to generate an immune response and had made such constructs. We knew from experience we had with other pox viral vectors how to administer said pox viral vector, and what an immunologically sufficient response was.

11. As shown by Exhibit "B", our ultimate goal was to use these pox virus vectors in humans.

12. Although the human testing occurred subsequent to the August 11, 1993 filing date of Spitler et al., we had generated recombinant viral vector vaccines for the prevention and treatment of prostate cancer. Spitler in contrast had not even made a PSA construct and provides no experimental results.

13. Thus, Spitler taught far less than we had already accomplished in the United States prior to its filing date.

14. We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information are believed to be true, and further that these statements are made with the knowledge that willful false statements and the like so

made are punishable by fine or imprisonment, and that such willful false statements may jeopardize the validity of the application or any patent that issues therefrom.

Date

Jeffrey Schlom

4-19-00

Date

Dennis Panicali

QUARTERLY PROGRESS REPORT

CONTRACT # NO1-CB-21154-02

PRODUCTION OF RECOMBINANT VACCINIA VIRUS EXPRESSING PROSTATE SPECIFIC ANTIGEN (PSA)

I. PLASMID INSERTION VECTORS

The gene encoding prostate specific antigen (PSA) has been inserted into two of Therion's plasmid vectors for recombination into vaccinia virus. These vectors each contain the PSA gene under the transcriptional control of the vaccinia 40K promoter and the *E.coli lacZ* gene to allow selection of recombinant progeny, but differ in the vaccinia genomic insertion site and in the promoter controlling *lacZ*. The important features of the two plasmids are summarized in the table below:

Plasmid designation	Vaccinia Insertion Site	Promoter/ PSA gene	Promoter/ *lacZ* gene
pT119*	HindIII J (tk)	40K / PSA	BamF / *lacZ*
pT1001	HindIII M	40K / PSA	C_1 / *lacZ*

*provided by NCI as "pAbT4537PSA"

II. GENERATION OF RECOMBINANT VIRUSES

The plasmid vector pT119 was used to generate recombinant virus in the Wyeth vaccine strain background. Three independent isolates, designated vT119 A, B and C, were purified using a colorimetric assay for β-galactosidase performed on viral plaques *in situ*. Recombinant viruses, which coexpress β-galactosidase and the PSA gene, appear blue in the presence of a histochemical substrate for the enzyme (Bluogal). Blue plaques are picked, re-plated, and are again treated with BluoGal. After seven rounds of plaque purification, all progeny plaques were blue, indicating purity of each recombinant virus stock. Viral plaque size was small, and plaques were heterogeneous with respect to levels of *lacZ* expression. This heterogeneity and the number of rounds of plaque purification required for recombinant purification were very unusual in our experience with generating vaccinia recombinants. Therefore, we undertook the generation of an

alternative recombinant using plasmid pT1001, described above. Recombinant plaques have been isolated and purification of recombinants, designated vT1001, is underway. vT1001 recombinants will be compared to vT119 recombinants with respect to plaque size, virus yield, and PSA expression. Based on the results of these analyses, one of the two recombinants will be selected for the manufacture of clinical grade material.

III. ANALYSIS OF RECOMBINANT VIRUSES

Recombinant viruses vT119A, B and C were analyzed with respect to genomic structure by Southern analysis using PSA-specific and vaccinia-specific probes. PSA protein expression was analyzed by Western analysis using polyclonal antibodies specific for PSA. The results were identical for the three isolates: each recombinant genome contains the PSA gene inserted into the vaccina HindIII J region, as expected, and each recombinant expresses PSA. Details of these analyses are given in the report on Genomic and Protein Expression Analysis dated

Genomic and protein analysis of alternative recombinant vT1001, discussed above, is expected to be completed by - If this recombinant is judged superior to vT119 for product manufacture, details of genomic and protein expression analysis will be submitted to NCI. Production of a Master Virus Stock for vaccine manufacture will begin in January. All work is on schedule.

EXHIBIT "B"

C. PSA/vaccinia

1. Generation and analysis of virus seed stock
2. Product manufacture
3. Testing/final reports
4. Master file preparation